REMARKS

Claims 104-109, 113-118, 125-137 and 140-144 are pending. Various typographical errors have been corrected in the specification. Claims 125 and 140 have been canceled. Claims 104-105, 113-115, 118, 126, 128, 130-137 and 141-142 have been amended. Claims 145 and 146 have been added. After entry of the above amendments, claims 104-109, 113-118, 126-137, 139-144 and 145-146 will be pending in the application. Applicants submit that these amendments do not add new matter, as discussed below.

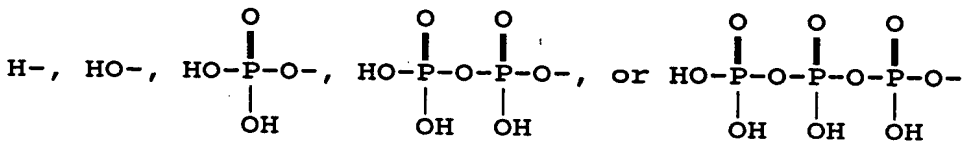
Claims 113, 126, 128, 130 and 134-137 have been amended to change the dependency on canceled claim 125 to new claim 145. Claim 105 has been amended to recite that the moiety A "consists of biotin or iminobiotin", instead of "comprises biotin and iminobiotin", as suggested by the Examiner. Claim 114 has been amended in the same manner as claim 105. Claim 115 has been amended to delete the word "comprises" and to specify that the listed polypeptides are part of a Markush group. Claim 118 has been amended to mirror the changes made in claim 109 in a prior amendment. Claims 126, 128, 130, 134-137 and 142 have been amended to delete the word "sequence" or "sequences" and to insert the word "nucleic acid" or "nucleic acids", where applicable. For proper antecedent basis, claims 130-133 have been amended to delete the word "bacterium" and to replace it with "microorganism". Claim 137 has been amended to clarify that the moiety A has been attached to the 5-position of the uracil.

New claim 145 has been added to replace canceled claim 125. New claim 145 corresponds to canceled claim 125, except

for (i) the insertion of the words "the steps of (a)" after the word "comprises" on line 2 and the term "(b)" after the word "and" and before the word "detecting" on the next to the last line; (ii) the insertion of the "z" that was omitted on the 3rd ribose when claim 125 was amended in the Response dated June 19, 1992, as noted by the Examiner; (iii) the correction of the location of one of the brackets in the figure; (iv) the placement of the letters m, n and p in the figure on the right hand side of the brackets instead of the left hand side of the brackets; (iv) the specification that the target is a nucleic acid; (v) the insertion of the statement that the A moiety is capable of producing a detectable signal when the compound is incorporated into a double-stranded ribonucleic acid or deoxynucleic acid duplex; and (vi) the replacement of the description that the moiety A "consists of" at least three carbon atoms with the description that the moiety A "comprises" at least three carbon atoms. Support for these changes can be found, e.g., on p. 7, line 9 to p. 8, line 14 and p.25, line 1 to p. 26, line 12 of the specification.

New claim 146 has been added to replace canceled claim 140. New claim 146 corresponds to canceled claim 140, except for (i) the cancellation of the dependency on claim 125 and the re-writing of the claim in independent form (since claim 125 was directed to a polynucleotide whereas claim 140 was directed to a nucleotide); (ii) the insertion of the term "the steps of (a)" and the terms "(b)", "(c)" and "(d)" before the appropriate method steps and the replacement of the appropriate commas with semicolons; (iii) the deletion of the phrase:

"each of x, y and z represents



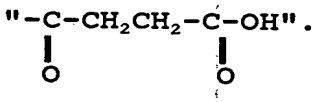
and in which x or"; (iv) the replacement of the words "are reacted to" with the word "together"; (v) the limitation of the claim to the 3',5' and 2',3' cyclic monophosphate moieties; (vi) the replacement of the description that the moiety A "consists of" at least three carbon atoms by the description that the moiety A "comprises" at least three carbon atoms; (vii) the deletion of the statement that A is capable of producing a detectable signal when the compound is incorporated into a double-stranded ribonucleic or deoxyribonucleic acid duplex and does not interfere substantially with the characteristic ability of the nucleotide to hybridize with the target and insertion of the statement that "A represents at least one component of a signalling moiety" ; and (viii) the changing of the last step from "identifying the same" to "detecting said compound in said fragments", for increased clarity. Support for the cyclic moiety is found at p. 40, lines 11-16 of the specification. The 3',5'-cyclic monophosphate, in which x and y are reacted to form a cyclic moiety, is well known to one skilled in the art, as is the 2',3'-cyclic phosphate. Support for "A" moieties which comprise an indicator molecule is found at p. 9, lines 6-10; p. 9, line 32 to p.10, line 2; and p.22, lines 15-17 of the specification. Specifically, these disclosures teach that the probe (i.e., the "A" moiety) may react specifically with chemical reagents to provide a detection system. It would be readily apparent to one skilled in the art that in order for "A" to react directly with chemical reagents and thereby provide a detection system, "A" itself would be capable of detection. At

page 22, one embodiment is disclosed wherein biotin- or iminobiotin-containing nucleotides may be radiolabelled on the "A" moiety.

1. Informalities

The Examiner has indicated that the application contains numerous errors. The Examiner requests that the application be reviewed and that errors be corrected without adding new matter.

Applicants have reviewed the disclosure and have amended the obvious spelling errors as requested by the Examiner. Applicants have also amended the obvious typographical error in the structure for NAGE-iminobiotin on p. 20, line 18. These amendments do not constitute new matter. Applicants have also amended the structure on page 12, line 27 of the specification to read



This amendment does not add new matter. The original structure on page 12, line 27 of the specification was obviously meant to represent a succinyl group, a hapten derived from succinic anhydride, in the same way the phthaloyl group on page 12, line 32 is derived from phthalic anhydride. Both of these anhydrides are commonly used to derivatize biomolecules. The specification teaches on p. 15, lines 23-28 that anhydrides can be used as a source of the moiety A.

2 and 3. Rejection under 35 U.S.C. § 112, first paragraph

The specification is objected to under 35 U.S.C. § 112, first paragraph, as failing to provide an enabling disclosure. Claims 140 and 141 are rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth in the objection to the specification.

Applicants have canceled claim 140 in the present application and replaced it with new claim 146, thereby rendering the objection and rejection moot. Claim 141 has been amended to depend on new claim 146. Applicants submit that this rejection has been overcome.

The Examiner notes that it appeared that applicants possibly intended that "H-" be one of the options for "x" as given in claim 140, line 25. The Examiner states that there is a lack of any enablement of such a 5' terminus in the instant disclosure. As noted above, Applicants have canceled claim 140, thereby rendering this rejection moot. New claim 146, which replaces claim 140, does not specifically recite "H-" as one of the options for "x". Therefore, this rejection does not apply to new claim 146. Thus, Applicants submit that this rejection has been overcome and that new claim 146 and amended claim 141 are in condition for allowance.

The Examiner points out that claims 140 and 141 cite structures where the "z" moiety is mono-, di-, or tri-phosphate. The Examiner states that such structures lack enablement in the instant specification. Applicants have canceled claim 140, thereby rendering this rejection moot. New claim 146, which replaces claim 140, does not contain mono-, di-, or tri-

phosphates as one of the options for "z". Therefore, this rejection does not apply to new claim 146. Claim 141 has been amended to depend on new claim 146. Thus, Applicants submit that this rejection has been overcome and that new claim 146 and amended claim 141 are in condition for allowance.

4. Rejection under 35 U.S.C. § 112, first paragraph

Claims 104-109, 114-118, 125-137 and 140-144 are rejected under 35 U.S.C. § 112, first paragraph, on the grounds that the disclosure is enabling only for claims limited to "A" moieties consisting essentially of either iminobiotin or biotin.

The Examiner has rejected all of the claims, including claim 105, because of the open claim language "comprises" cited in line 2 of claim 125 as well as line 2 of claim 105. The Examiner states that the clear and concise enablement of "A" beyond said iminobiotin and biotin is lacking due to the citation of several of the structures given in the specification on page 12 as examples of enablement beyond iminobiotion and biotin.

With respect the structure on page 12, at line 27, the Examiner notes that the left hand structure is shown as having two unattached bonds available for attachment to the linker or nucleoside base. The Examiner states that this two bond example of "A" prevents a clear and concise enablement of the moiety "A" since there is no discussion of a two bond attachment to "A" from either the linker of nucleoside base. Applicants submit that this defect in the specification is clearly due to a typographical error. As noted above, the original structure on page 12, line 27 of the specification was obviously meant to

represent a succinyl group, a hapten derived from succinic anhydride, in the same way the phthaloyl group on page 12, line 32 is derived from phthalic anhydride. Both of these anhydrides are commonly used to derivatize biomolecules. Also as mentioned above, the specification teaches on p. 15, lines 23-28 that anhydrides can be used as a source of the moiety A. Thus, one of ordinary skill in the art would have realized that this was a typographical error.

With respect to the aromatic structures on page 12, line 20, the Examiner queries how those structures are enabled versus other aromatic structure. The Examiner states that a problem with aromatic "A" moieties, i.e., intercalation, is stated on page 13, lines 1-3, and notes that it is never resolved how the aromatic structures on page 12, line 20, for example, are enabled versus other aromatic structures. The Examiner asks what criteria of intercalation should be used to resolve the issue and notes that no test is discussed in the specification. The statement referred to by the Examiner reads as follows:

> Moreover, since aromatic moieties tend to intercalate into a base-paired helical structure, it is preferred that the moiety A be nonaromatic.

First of all, Applicants submit that the metes and bounds of the moiety A are definite and clearly defined in the claims. As to the Examiner's question regarding what criteria of intercalation should be used, Applicants point out that there is no statement in the specification that intercalation represents a "problem" that must somehow be overcome. The specification merely asserts that non-aromatic linkers are "preferred" over aromatic linkers. The reference to intercalation is merely an explanation for this preference. As

to the Examiner's statement that no test is discussed in the specification, Applicants note that simple tests existed at the time of filing of the specification to determine whether intercalation had occurred. For example, the method of detection of UV absorbance or hyperchromicity of a polynucleotide, which is referred to on p. 29, lines 32-35 of the specification in reference to studies of denaturation and renaturation profiles, was well known in the art as a means for determining intercalation. Since this method was well known in the art, there was no need for the specification to specifically describe the test in reference to intercalation.

The Examiner also refers to the six "essential criteria" listed on page 9, line 1, through page 10, line 19 as further support for undue experimentation with respect to "A" moieties. The Examiner asserts that

> "[s]atisfaction of all these criteria is clearly undue experimentation, especially since the practice of satisfying these criteria is not clearly and concisely enabled in the instant specification".

The practice of satisfying these criteria is clearly and concisely enabled with respect to biotin and iminobiotin. It is well established that the provision of even a single working example can support a broad, basic invention. To be enabling under 35 U.S.C. §112, a specification must contain a description that enables one skilled in the art to make and use the claimed invention. Atlas Powder Co. v. E.I. du Pont de Nemours & Co., 750 F.2d 1569, 1576 (Fed. Cir. 1984). That some experimentation is necessary does not constitute lack of enablement. Moreover, it is not necessary that the specification test all the embodiments of the invention. In re Angstadt, 537 F.2d 498, 502 (CCPA 1976); what is necessary is that he provide a disclosure sufficient to enable one skilled in

the art to carry out the invention commensurate with the scope of his claims. For Applicants' modified nucleotides, that means disclosing how to make and use enough modified nucleotides to justify grant of the claims sought. Applicants have done so.

The six "essential criteria" listed in the specification are

(1) The modified compound must contain a substituent or probe that is unique, i.e., not normally found associated with nucleotides or polynucleotides.

(2) The probe must react specifically with chemical or biological regents to provide a sensitive detection system.

(3) The analogs must be relatively efficient substrates for commonly studied nucleic acid enzymes, since numerous practical applications require that the analog be enzymatically metabolized, e.g., the analogs must function as substrates for nucleic acid polymerases.

(4) The detection system should be capable of interacting with probe substituents incorporated into both single-stranded and double-stranded polynucleotides in order to be compatible with nucleic acid hybridization methodologies.

(5) The physical and biochemical properties of polynucleotides containing small numbers of probe substituents should not be significantly altered so that current procedures using radioactive hybridization probes need not be extensively modified.

(6) The linkage that attaches the probe moiety should withstand all experimental conditions to which normal nucleotides and polynucleotides are routinely subjected, e.g., extended hybridization times at elevated temperatures, phenol and organic solvent extraction, electrophoresis, etc.

Applicants submit that, given the teachings of the specification and general knowledge, one of ordinary skill in the art at the time of filing of the application would have known how to meet the six criteria, with little or no experimentation: (i) one skilled in the art would know how to meet the first criterion (the group "A" is not normally associated with nucleotides or polynucleotides) since the structure of nucleotides and polynucleotides generally found in nature is known; (ii) the second criterion is met by choosing the moiety "A" from those compounds known to constitute ligands (or one partner of a specifically-binding pair of molecules), as taught in the specification, since the moiety "A" must react or bind with the detection system to be used to detect the moiety "A" (see, e.g., p. 12, lines 6-35 of the specification); (iii) the third criterion (substrate for polymerases) is satisfied by the placement of the moiety A on the 5-position of pyrimidines, 7-position of deazapurines or 8-position of purines, since nucleotides modified at these positions are known to be substrates for enzymes and are taught to be such in the specification (see, e.g., p. 9, lines 24-26 of the specification); (iv) the fourth criterion (availability of "A" for interaction with a detection system) is satisfied by both the above-mentioned position of the moiety A (e.g., the 5-position of pyrimidines, 7-position of deazapurines or 8-position of purines) and the nature of the moiety A (i.e., at least three carbon atoms) (see, e.g., p. 12, lines 1-35 of the specification); (v) one of ordinary skill in the art would know how to meet the fifth criterion (the physical and biochemical properties of polynucleotides containing small numbers of probe substituents should not be significantly altered so that current procedures using radioactive hybridization probes need not be extensively modified) by conducting routine tests to determine

the number of modified nucleotides which can be used in the method of the invention, e.g., the denaturation, renaturation, and hybridization tests set forth on p. 29, line 11 to p.30, line 56 of the specification; (vi) one of ordinary skill in the art would know how to meet the sixth criterion (the linkage that attaches the probe moiety should withstand all experimental conditions to which normal nucleotides and polynucleotides are routinely subjected) since one skilled in the art would know which bonds and structures are stable under the conditions used to isolate nucleotides and conduct hybridizations, etc.

With respect to the structures on page 12 of the specification, the Examiner asserts that Applicants provide no discussion of how the lipoyl group on line 27, right column, meets any of the criteria established for "A". Applicants note that this group is not normally associated with nucleotides. Thus, the first criteria is satisfied. Applicants also note that this structure is an example of one class of specific-binding pairs: enzyme-substrate complexes. Lipoic acid and lipoamide are both substrates for lipoamide dehydrogenase. Lipoamide dehydrogenase binds to lipoyl groups tightly enough to enable purification of the enzyme by affinity chromatography when the lipoyl group is bound to a solid support. See W.H. Scouter et al. Biochem. Biophys. Acta 309:521-524 (1973). Lipoamide is also a cofactor for several other enzymes, which could also serve as binding partners for a lipoyl-modified nucleotide. Thus, the second criteria is satisfied. The rest of the criteria are also easily satisfied, for the reasons set forth above.

In sum, the test for the suitability of a particular moiety "A" for the claimed method would be routine. Attachment

of the moiety "A" can be carried out quickly, e.g., by mercuration and amino-alkylation of the nucleotide and subsequent attachment of "A" group, as set forth in the specification. The ability of the modified nucleotides containing the moiety A to be incorporated into a polynucleotide, if desired, is easily confirmed, e.g., by using the modified nucleotide in nick-translation, gap-filling, permeabilized cells or in a nuclear replication system (see, e.g., p. 21, line 11 to p. 22, line 13 of the specification) and monitoring the incorporation, e.g., by affinity chromatography or immunoprecipitation (see p. 22, line 15, to p. 23, line 7). The number of modified nucleotides to use which contain the moiety A could easily be determined, e.g., by conducting denaturation and renaturation experiments (discussed above) and detection of the moiety A carried out under conditions appropriate to the detection system used (see, e.g., p. 27, lines 26-34 of the specification). Finally, the specificity of the probe could be determined easily by comparison with a control system. For example, the specification teaches that polytene chromosomes could be used as a test system for establishing the efficacy of the probes using modified nucleotides by using probes with known mapping sites (p. 33, line 33 to p. 34, line 9). The above methods were routine at the time of filing of the application.

Adequate screening methods have been held to overcome the "undue experimentation/enablement" rejection where the practitioner is attempting to bridge the gap between the embodiments actually disclosed and exemplified in the application and the scope of the protection which the application is entitled to based on the state of the art. See, e.g., Tabuchi v. Nubel, 559 F.2d 1183, 194 U.S.P.Q. 521 (CCPA

1977) and In re Wands, 858 F.2d 731, 8 U.S.P.Q.2d 1400 (Fed. Cir. 1988).

Applicants submit that the chemical attachment to a nucleotide of group "A" is enabled by the specification and knowledge of one of ordinary skill in the art. Applicants submit that the teachings of the current application enable one skilled in the art to attach not only biotin, but any other detectable group, to a nucleotide and then detect the nucleotide with the appropriate detection system. Thus, it is Applicants position that the "A" moieties are enabled beyond the exemplified biotin and iminobiotin.

In light of the above remarks, Applicants respectfully request that the rejection of claims 104-109, 113-118, 125-137 and 140-144 under 35 U.S.C. § 112, first paragraph for failure to provide an enabling disclosure for "A" moieties other than biotin or iminobiotin be reconsidered and withdrawn.

5. Rejection under 35 U.S.C. § 112, second paragraph

Claims 104-109, 114-118, 125-137 and 140-144 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner states that the structure in claim 125 is vague and indefinite because the third ribose moiety lacks the "z" group at the 2' position as given on the other ribose moieties as well as in the previous claim 125 prior to the amendment filed 6/24/91. Applicants have canceled claim 125 and

have replaced it with new claim 145. New claim 145 includes the "z" group at the 2' position, as requested by the Examiner. Applicants submit that this rejection has been overcome.

The Examiner states that the moiety "H-HO-" as one of the represented options for "x, y and z" in Claim 140 is vague and indefinite as to what is meant. This was clearly a typographical error for the moieties "H-, HO-". Applicants have canceled claim 140 and added claim 146 in its place. Claim 146 does not give the moiety "H-HO-" as one of the represented options for "x, y and z". Applicants submit that this rejection has been overcome.

The Examiner also queries what are the metes and bounds of the "A" moiety as instantly claimed. Applicants note that pending claims 125 and 140 have been canceled and new claims 145 and 146 have been added. Applicants note that the questioned terms are not currently used in new claims 145 and 146. Applicants submit that the metes and bounds of the "A" moiety as claimed in new claims 145 and 146 are definite and clearly defined. For example, in claim 145, it is specified that the moiety A (i) represents at least one component of a signalling moiety capable of producing a detectable signal when the compound is incorporated into a double-stranded ribonucleic or deoxyribonucleic acid duplex; (ii) comprises at least three carbon atoms; (iii) is attached to the nucleotide directly or through a linkage group, the linkage group not interfering substantially with the characteristic ability of B to hybridize with said target or of A to produce a detectable signal; and (iv) is attached to the 8-position of a purine, the 7-position of a deazapurine, or the 5-position of a pyrimidine. In claim 146 it is specified that A (i) represents at least one component

of a signalling moiety, (ii) comprises at least three carbon atoms; and (iii) is attached to the nucleotide directly or through a linkage group, the linkage group not interfering substantially with the characteristic ability of A to form a detectable complex with said polypeptide. Thus, Applicants submit that the metes and bounds of the moiety "A" are clear and definite.

The Examiner objects to the citation of the target as "nucleic acid sequence" in Claim 126, line 3 and in several other claims also. The Examiner states that this is a mathematical representation of a polynucleotide and not a composition. The Examiner requests clarification of the claim language. Applicants have deleted the word "sequence" from "nucleic acid sequence" in Claim 126, line 3 and have similarly deleted the word sequence(s) from other claims in which it appeared, as discussed above. Applicants submit that this rejection has been overcome.

The Examiner states that Claim 140 is vague and indefinite in that a "cyclic moiety" is cited in line 28, followed by a depiction of a structure which is not cyclic. Applicants have canceled claim 140 and replaced it with new claim 146. The language in new claim 146 has been rewritten to avoid the citation of a cyclic moiety followed by a depiction of a structure which is not cyclic. Applicants submit that this rejection has been overcome.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants submit that the instant application is in condition for allowance. Favorable reconsideration and an action passing this case to issue are therefore respectfully requested.

Respectfully submitted,

MORGAN & FINNEGAN

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Mary J. Morry
Reg. No. 34,398

Mailing Address:
MORGAN & FINNEGAN
345 Park Avenue
New York, New York 10154
(212) 415-8500
(212) 751-6849 Telecopier